authentic corticosterone was available for comparison. Corticosterone, however, is reported to have only a slight eosinopenic effect, which would not contribute appreciably to the biological activity detected in these ascitic fluid extracts.

It may be concluded, therefore, that almost all of the biological activity observed is due to the content of 17-hydroxycorticosterone, and that this varies in different samples of ascitic fluid from zero to about 5 μg. per 100 ml. Cortisone itself does not contribute to the biological activity in significant degree.

It is, of course, clear that one cannot assume that the hormone content of ascitic fluid is necessarily the same as that of circulating blood. Ascitic fluid may, however, be regarded as representing a form of extracellular fluid. Its hormone content may be indicative of the adrenal hormone content of extracellular fluid in general, a content which must be of more direct importance to living cells than is the hormone content of the blood. Our inability to demonstrate any hormonal activity in the two samples from patient Fr raises the possibility that conditions may sometimes exist in which adrenal cortical hormone diffuses with difficulty from the blood into the extracellular fluid. Such a state could account for the fact that rheumatoid arthritic tissues and joints respond promptly to cortisone or 17-hydroxycorticosterone therapy, although all the evidence suggests that adrenal cortical function is normal in this disease. While this suggestion is purely speculative at the present time, further investigation of the hormonal content of human ascitic fluid should prove of considerable interest, in so far as it is representative of an extracellular fluid.

Summary

The adrenal cortical hormone content of human ascitic fluid has been measured biologically, using the power of such hormones to lower the level of circulating eosinophil cells in adrenalectomized mice.

Of ten samples of ascitic fluid tested, five showed activity equivalent to between 3 and 5 μg. of cortisone per 100 ml. In two samples from one patient no activity at all could be detected. In the remaining three activity was equivocal and less than 1.5 μg. per 100 ml.

17-hydroxycorticosterone (compound F) was detected by chromatographic analysis in all four samples in which it was sought, but cortisone was found only as a faint trace in a single extract. The biological activity is apparently due almost entirely to the 17-hydroxycorticosterone, only a negligible part being due to cortisone.

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PIPERAZINE IN THE TREATMENT OF THREADWORMS IN CHILDREN

REPORT ON A CLINICAL TRIAL

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The successful use of piperazine in the treatment of threadworms was first recorded by Mouriquand, Roman, and Coisnard (1951) and later by Turpin, Cavier, and Savaton-Pillet (1952). Mouriquand et al. used piperazine hydrate in solution, while Turpin et al. chose piperazine diphenyl-acetate in the form of glutenized granules. In most instances oral dosage was supplemented by the use of piperazine suppositories. The clinical trials carried out by both groups of workers were preceded by successful preliminary experiments upon the mouse Aspiculuris or Syphacea. In the clinical trials Mouriquand et al. reported a high proportion of cures in 18 patients, while Turpin et al. recorded one definite failure out of 41 patients.

We ourselves decided to carry out clinical trials following successful experiments with piperazine hydrate against Aspiculuris in the mouse (Standen, 1953). As a comparative standard, gentian violet was also included, and, in addition, diphenan, which has achieved considerable popularity on account of its lack of toxic side-effects. Laboratory tests had failed to demonstrate oxyuricidal properties in diphenan. These results were in keeping with the clinical findings of Miller and Choquette (1950), Beaver and Young (1950), Dowsett and Brown (1953), Jung and Beaver (1953). In contrast, Killingsworth et al. (1952), using diphenan in conjunction with hexylresorcinol enemas, reported 77% cures, while Bumbalo et al. (1953) reported 30% cures with diphenan alone. Killingsworth et al. and Van Someren (1939) both quote Schulemann (1920) as stating that diphenan breaks down in the small intestine to form para-benzylphenol, and that this kills oxyurids in 5–10 minutes at 1:4,000 dilution. In fact, Schulemann was working with "Strudelwurmen," which are free-living turbellarians and not in any way related to oxyurids in particular nor to nematodes in general. It is considered that this mistranslation of Schulemann's work should be recorded, since it appears to be in danger of perpetuation in the literature.

Clinical Tests

The material used in the tests consisted chiefly of children admitted to hospital for medical or surgical reasons, and these were identified as cases of Enterobius infection by microscopical examination of adhesive "cellophane" swabs of the perianal area. Swabbing was carried out in the morning before the children were bathed or used the toilet, and was repeated for seven days unless a positive result was obtained earlier. In addition, previously established cases of oxyuriasis were included. For purposes of comparative drug effect the children were treated in four groups, each group receiving piperazine, gentian violet, diphenan, or a lactose placebo.

Treatment

(a) *Piperazine.*—Used in the form of piperazine hexahydrate embodied in a flavoured syrup. The children receiving piperazine were divided into two groups, one receiving 100 mg. and the other 250 mg. per day per year of life, the drug being given in divided doses, morning and evening. Treatment continued for seven days, and a second similar course was given after seven days' rest.

(b) *Gentian Violet.*—Given as sugar-coated pills at a dose level of approximately ⅙ gr. (11 mg.) daily per year of life in divided dosage for seven days and repeated after seven days.

(c) *Diphenan.*—Given as whole or fractions of plain 500-mg. tablets. Owing to difficulty in subdivision of tablets to suit each year of age, the children were arranged in broad age groups. Inevitably this meant a range in dosage from approximately 100–300 mg. t.d.s. per year of life in the lower dosage group and approximately 200–600 mg. t.d.s. for those at higher dosage. The course of treatment was for seven days, and was repeated after seven days' rest.

(d) *Lactose Placebo.*—A control group was considered highly desirable in order to determine the degree of spontaneous cure. The placebo was given morning and evening in two courses of seven days as with the other drugs.

Follow-up

Swabbing of the perianal area with adhesive cellophane was begun 14 days after completion of the second treatment period, and was continued each morning for seven successive days. Any single swab showing one or more ova indicated failure to cure, while seven successive negative swabs formed the criterion for cure.

Hygienic Precautions

In most instances the children received treatment as outpatients, and it was considered unrealistic to expect parents to adhere uniformly to strict hygienic precautions. Consequently, beyond instruction concerning general cleanliness, no special precautions were imposed. Thus the trials were carried out under conditions that would normally prevail in an average household.

Toxicity and Side-effects

(a) *Piperazine.*—During the first quarter of the century piperazine was widely used in the treatment of gout and allied conditions, but it has fallen into disuse because of ineffectiveness in these complaints. Consequently many reports have been published concerning its use in this respect and no important toxic effects have been recorded. Absence of toxicity has been reported by numerous authors, notably Frankel (1921), Gadamer (1924), Fayard (1949),

Mouriquand *et al.* (1951), and Cavier (1953). Recent pharmacology and toxicity studies in laboratory animals (De Beer, unpublished) failed to elicit any significant toxic effects. In our experience during clinical trials only one case showed intolerance. At a dose level of 58 mg./kg. per day, this child complained of loose stools. When treatment was discontinued the diarrhoea ceased immediately. The drug was, in fact, found to be extremely well tolerated even up to dose levels of 150 mg./kg. daily. This higher dose relates to treatments of ascariasis, which will form the subject of a further communication.

(b) *Gentian Violet.*—Out of 20 cases treated two complained of abdominal pain and one experienced nausea and vomiting during the first week of treatment. Otherwise no side-effects were reported.

(c) *Diphenan.*—Treatment with diphenan produced no toxic side-effects.

Results

Comparative Results.—At 100 mg. per day per year of life piperazine hydrate gave a lower cure rate than gentian violet at ⅙ gr. (11 mg.) daily per year of life (see Table)—that is, 54% and 70% respectively. At the higher dose level of 250 mg. per day per year of life the cure rate for piperazine hydrate was much enhanced, giving 83% cures out of 42 cases treated. The efficiency of diphenan at both dose levels (see Table) closely resembled that of the lactose placebo—that is, 17% out of 23 cases and 19% out of 16 cases respectively.

Analysis of Piperazine Results.—It was appreciated that considerable weight variation occurred within each age group tested, and analysis of the total results of piperazine hydrate tests at both dose levels (see Fig.) shows that on a weight/dose/cure ratio a cure rate of 97% was obtained where the daily dose exceeded 50 mg./kg. body weight.

Chart showing relationship of dose of piperazine hydrate in mg./kg. per day to cure in 69 cases of oxyuriasis.

Conclusions

The results obtained from the clinical trial of piperazine hydrate, gentian violet, diphenan, and a lactose placebo as control showed that while gentian violet had a marked oxyuricidal effect (70% cures) at a dose level of ⅙ gr. (11 mg.) daily per year of life, this was well surpassed by piperazine hydrate syrup when the dose level was in excess of 50 mg./kg. per day, and the cure rate reached 97%. Though diphenan gave a cure rate of 17% at a dosage of 100–600 mg. daily per year of life, this apparent oxyuricidal effect was discounted by the fact that 19% cure was obtained with a lactose placebo control. The latter indicated that a spontaneous clearance of threadworms may be expected in approximately 19% of infections. Thus piperazine hydrate syrup proved to be the most effective, while producing negligible side-effects; gentian violet was less effective, and was also undesirable on account of its nauseating effects and staining properties; diphenan was ineffective. It is concluded that piperazine hydrate, at a dose level of 50–75 mg./kg. per day, is the drug of choice for the treatment of oxyuriasis in children.
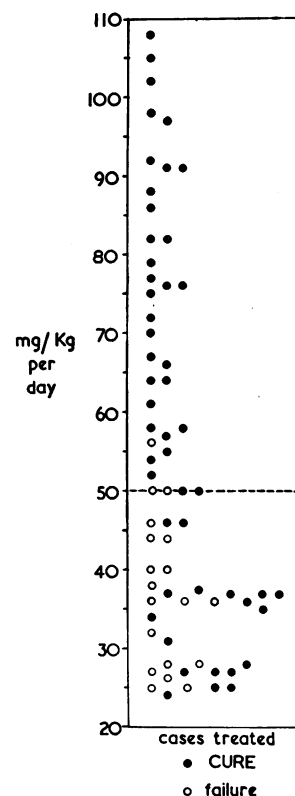
Table showing the Comparative Effectiveness of Drugs Used in Clinical Trials Against Enterobius vermicularis Infections in 136 Children

Drug	Daily Dose per Year of Life	Age Range	Total No. Treated	Cases Followed Up			
				Total No.	No. of Cures	No. of Failures	% Cures
Piperazine hydrate	100 mg.	1½–11 3 adults	44	35	19	16	54
	250 mg.	1½–13 2 adults	64	42	35	7	83
Gentian violet	1/6 gr. (11 mg.)	2½–12	38	20	14	6	70
Diphenan	100–300 mg.	1½–11	34	22	4	18	18
	200–600 mg.	11	7	1	—	1	—
	Combined results	1½–11	41	23	4	19	17
Lactose placebo	—	2–7	36	16	3	13	19

Summary

In clinical trials of piperazine hydrate, gentian violet, and diphenan against oxyuriasis in 136 children, piperazine hydrate syrup and gentian violet showed marked oxyuricidal properties, while diphenan was inactive.

Owing to lack of toxicity and side-effects, to ease of administration, and to its excellent therapeutic properties, piperazine hydrate syrup at a dose level of 50–75 mg./kg. per day is considered to be the drug of choice for oxyuriasis in children. Of 31 children treated at levels greater than 50 mg./kg. daily 97% were cured.

The use of suppositories is unnecessary.

Spontaneous cure may be expected in 19% of cases of oxyuriasis.

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EXPERIMENTAL CHEMOTHERAPY OF OXYURIASIS

BY

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The two drugs most commonly used in the treatment of oxyuriasis are gentian violet and diphenan. From time to time claims of efficacy in clearing threadworms have been made for other substances, notably aluminium 8-hydroxyquinoline (" nyxolan "), N-*iso*amylcarbaminic acid-3-methyl-6-*iso*propyl phenyl ester (" egressin "), and garlic preparations. Owing to the length of the life cycle and the possibilities of auto-infection, properly controlled clinical trials against *Enterobius (Oxyuris) vermicularis* are extremely complicated. The eight weeks or so required for diagnosis, treatment, and follow-up is longer than children usually remain in hospital, and any trials started under the control and supervision of the medical and nursing staff must be continued with the co-operation of the parents. The difficulties inherent in such an arrangement probably account for the relatively few occasions upon which adequate clinical trials have been made with potential oxyuricides. Obviously some

laboratory method of screening drugs of this kind is desirable, so that these cumbersome clinical trials may be initiated only when justified by experimental evidence.

As no laboratory animal has been found that will act as host to *E. vermicularis* the oxyurids of rodents have been employed for chemotherapeutic trials. The laboratory evaluation of egressin was carried out using *Passalurus ambiguus* in the rabbit (Eichholtz and Hotovy, 1950) ; *Aspiculuris tetraptera*, oxyurid of the mouse, was used for study of the oxyuricidal properties of antibiotics (Wells, 1951a, 1951b, 1952a, 1952b) ; *Syphacia obvelata* in the mouse was used by Chan (1952) for screening a number of potentially active substances, while Turpin *et al.* (1952) tested piperazine against both *Aspiculuris* and *Syphacia*. Mouriquand *et al.* (1951) used piperazine hydrate against *A. tetraptera* in the mouse.

A. tetraptera occurs naturally in the caecum and rectum in many laboratory strains of white mice. For the experimental work here described it was decided to use such natural infections with this parasite and to carry out a series of tests on substances of reputed activity against human threadworms. Tests on certain closely related substances were also to be included. By this means it was hoped to determine whether tests against *A. tetraptera* in the mouse would provide valid indications of activity against *E. vermicularis* in man and to demonstrate substances worthy of clinical trials.

Material and Method

White mice 11–12 weeks old showing ova of *Aspiculuris* in the faeces were segregated and used for drug-testing. Necropsy of untreated mice showed a range of 2–120 worms per mouse. Because of the relatively small number (10–20) of mice that could be used in each test it was considered that this range in numbers of worms was too large to warrant valid statistical deductions to be drawn on the basis of reduction in worm numbers following treatment. Therefore the degree of activity of a drug was estimated in relation to the dose required to clear the worms from all or a proportion of the mice treated. The drugs were given orally by stomach tube as a solution or suspension in water or liquid paraffin. The animals were dosed either once or twice daily for five consecutive days. According to the availability of infected mice, 10–20 animals were used for each test. Faecal examinations were made at six days and necropsy at 12 days after completion of treatment. In some instances necropsy was carried out at six days after treatment without prior faecal examination.

To facilitate worm counts the rectum and caecum of each mouse were removed at necropsy and placed in Bouin's fixative. Material fixed in this way could be stored and examined when convenient. At examination this portion of the gut was slit open under water and the contents were removed gently with a camel-hair brush. The inside of the examination dish was painted black, and the worms, now stained bright yellow, were easily seen and counted against this background.

Results

Table I shows the relative efficiency of a number of reputed oxyuricides in clearing mice of *Aspiculuris*. None of the drugs used, except perhaps oil of garlic, was particularly toxic* at the dosages employed, and only two, gentian violet and piperazine hydrate, produced any marked worm clearance. In seven other instances partial clearances were obtained, but four of these (Nos. 4, 6, 10, and 13)

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* All mice on test had been exposed to infection with *Schistosoma mansoni*. A proportion of the mouse mortality would result from this.